

ADVANCED GENETIC STRATEGIES FOR IMPROVING RICE YIELD

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Every crop breeding effort aims to increase production. Despite several advances, most worldwide breeding projects have traditionally focused on improving grain production potential, insect/pest and disease resistance, grain quality, and stress tolerance. Almost all rice breeding initiatives aim to increase grain yield. However, the value of this crop varies by area, country, and even within a country. Finding innovative ways to increase the genetic grain production potential of all kinds has significant ramifications. Rice breeders have improved crop yields significantly. The green revolution gave us semi-dwarf rice types, a new rice plant type, and hybrid rice. Conventional hybridization, ideotype and heterosis breeding, broad hybridization, genetic engineering, molecular marker-assisted breeding, and CRISPR-Cas9 are all possibilities for increasing yield potential. Pedigree is the most common breeding technique for enhancing rice, but hybrids and population improvement are also used. Many groups are still working to integrate biotechnology tools into breeding programs and balance budget allocation between conventional and innovative approaches. Modern technology, such as biotechnology, has recently increased agricultural productivity by improving crop yields and reviewing several genome editing methods to enhance rice production.

Keywords: Rice (*Oryza sativa* L.), genetic engineering, ideotype breeding, RISPR-Cas9.

INTRODUCTION

Rice (*Oryza sativa* L.) is the 2nd most extensively farmed grain globally, accounting for two-thirds of calorie consumption in Asia and one-third in Africa and Latin America. In terms of acreage and yield, it is also the planet's 2nd major food crop. It accounts for 90% of global output, is consumed by almost half of Asia's population (Virk *et al.*, 2004). Rice has made the most remarkable progress in functional genomics (Moin *et al.*, 2017). It has a minor genome compared to other cultivated grains. China, India, Indonesia, and the U.S. are the most consumed rice areas (Hegde *et al.*, 2013). Throughout West Africa and Madagascar, rice is the most widely consumed grain. It is gaining popularity in East, Central, and Southern Africa (Balasubramanian *et al.*, 2007). Rice farming is a recent phenomenon in Ethiopia. However, its culinary and economic worth is now generally recognized. Rice is one of the target commodities that has received adequate attention in supporting agricultural growth (Moncada *et al.*, 2001). Programs for crop breeding gather, induce and combine

desirable phenotypes across crop species to meet human needs (Fu *et al.*, 2015). Because of this, breeding efforts should focus on cultivars that minimize yield losses while boosting yields. Several breeding techniques can increase production potential.

- Conventional Breeding and Selection Procedures
- Wide Hybridization
- Ideotype (New plant type) Breeding
- Heterosis Breeding
- Molecular Marker-Assisted Breeding
- CRISPR-Cas9
- Genetic Engineering
- Genome Editing Technologies

Conventional breeding and selection procedures:

Genetically modified plants have been in use for hundreds of years, and they are still commonly used today. Early farmers realized that agricultural plants might be deliberately married or cross-pollinated in order to increase yields. It is a tried-and-true approach for finding high-yielding crop types. It has two sections. The first phase involves the cross-pollination of



various parents to produce diversity; step 2: Select desirable individuals based on field observations and yield testing. Rice yield potential has increased by 1% every year since the release of the 1st improved rice variety, IR8 (Peng *et al.*, 2000). The most prevalent rice breeding method is pedigree breeding. Rice is a self-pollinated crop; thus, recombination breeding involves crossing selected parents and selecting superior recombinants in successive generations for desired characteristics. Any worldwide literature review of rice breeding techniques usually places pedigree selection first. More than 85% of the Crop Science Society of America's released rice varieties are pedigree chosen. When several generations per year are possible, single-seed descent or modified bulk is employed to generate pure lines for agronomic evaluation swiftly. The best way to combine a variety's distinguishing qualities is to employ convergent improvement, which entails gradually adding constituent attributes. Pedigree methods increase quality and quantity when land/laboratory facilities and people are available.

Modified pedigree or mass pedigree selection is utilized when the selection environment cannot differentiate between promising and undesirable genotypes. The mass pedigree method involves combining five segregating generations from F₂ before performing the pedigree selection. Agronomically sound lines with excellent flooding tolerance were produced by IRRI utilizing standard breeding procedures (Khan *et al.*, 2015).

Ideotype (New Plant Type) Breeding: It was not long before the new plant-type paradigm in agricultural manipulation gained traction. Changing plant design through ideotype breeding has been proven to increase yield potential. To increase biological yield, high-yielding rice cultivars should be created (Yang *et al.*, 1996). A new rice ideotype (N.P.T.) with a 60% harvest index and enhanced photosynthesis was created by IRRI. A short growth cycle of 100 to 130 days was studied for this N.P.T. A rice plant with these characteristics might increase yields by 20% but at a higher cost and input.

Furthermore, many breeding lines with the desired ideotype have been developed (Khush *et al.*, 1995). It was impossible to achieve satisfactory yields using the first-generation novel plant type (N.P.T.) lines from tropical japonica due to low biomass output and poor grain filling. N.P.T. lines of Premier indica x enhanced tropical japonica have progressed (Peng *et al.*, 2008). Second-generation N.P.T. lines and Indica check variants outperformed first-generation N.P.T. lines. Using ideotypes and inter-subspecific heterosis, China's "super" rice breeding program has created many hybrids (F₁). These hybrids produced 12 t/ha in on-farm experiments, 8-15 percent more significant than the hybrid check variety. The assembly of the main elements of IRRI's N.P.T. design was credited with China's "super" hybrid rice success.

Chinese researchers propose combining this plant with heterosis breeding to generate super rice hybrids with 100 kg/ha yields. The plant stands at the height of 100 cm, with a 70 cm length of culm. The flag leaf is 50 cm long, while the other two are 55 cm. The panicle's top two leaves are long, and the second and third leaves of the flag have vertical leaf angles of 50, 100, and 200 degrees. Because it allows for developing novel crop varieties that are sensitive to fertilizers, irrigation, and other agricultural inputs, this method is critical for modernizing traditional agriculture. Many emerging nations may benefit from plant-type genes to increase food output.

Heterosis Breeding: Heterosis refers to hybrids outperforming their parents. Heterosis has long been used to boost agricultural production. Heterosis breeding, which exploits the hybrid vigor phenomena, has proved helpful in improving agricultural production potential. Heterosis refers to the hybrids' superior performance over their parents (Shull, 1914). This phenomenon has been used extensively in maize, pearl millet, onion, sorghum, cotton, etc. However, it is being used on rice and other self-pollinated crops. More than half of China's rice acreage is planted with hybrid rice, and other nations are developing and adopting hybrid rice technology. Chinese rice hybrids with a 10-15 percent yield advantage over best-inbred varieties did not enter the market until the mid-1970s when they were introduced in the United States. Tropical rice hybrids with similar yield advantages have been produced by the International Rice Research Institute (IRRI).

Wide Hybridization: Wide hybridization occurs when genes from diverse gene pools are exchanged across species. Plant gene pools are expanded by crossbreeding with wild species, intra-subspecific crossings, and weedy races, among other methods. Gene pools enhance a variety of factors, including yield. Avena sativa BC₂-BC₄ segregants Crosses of Avenasterilis yielded more grain than the grown recurrent parent (Lawrence *et al.*, 1976). Nine lines from this study had similar agronomic traits as the recurrent parent and produced 10-29 percent more grain across years and locations. A higher production potential was connected to better vegetative growth rates or early seedling vigor. Oryza rufipogon accessions from Malaysia surpassed farmed rice backcrosses by up to 18% (Xiao *et al.*, 1996). They found two QTL in wild species that boosted production. These QTLs have been used for breeding semi-dwarf cultivars.

molecular marker-assisted breeding: Both the Indica and Japonica genomes have been sequenced, giving breeders the resources; they need for marker-assisted breeding. In addition to S.S.R. markers, candidate gene markers are quickly being developed. Abiotic and biotic stress tolerance, cooking and nutritional quality, and yield are possible M.A.S. targets. Resistance gene pyramiding for diseases and pests. Disease and pest resistance genes commonly cross varietal lines. Transforming genes is difficult since they are recessive. The screening takes time, money, and a lot of field

area. If genes are intimately connected with molecular markers, transferring them from one varietal background saves time and money. An early molecular marker's existence or absence reveals the presence or absence of a target gene. The indirect selection might be enabled by using a molecular marker linked with the target gene as a "tag."

Pyricularia oryzae blights and *Xanthomonas oryzae* blight are two of the most severe and pervasive rice diseases. IRRI is employing molecular marker technology to create long-term disease resistance. To discover indicators linked to bacterial blight susceptibility genes, near-isogenic lines with single resistance genes have been very helpful. To validate RFLP marker-resistance gene co-segregation, segregating populations were used (Zheng *et al.*, 1995). If desired, it is also feasible to convert RFLP markers to PCR markers and then use them in the marker-aided selection process. The PCR markers were also used for hunting for genes that were resistant to bacterial blight, which was another goal of the study. As a result, Xa4, X5, Xa13, and Xa21 were merged (Huang *et al.*, 1997). Resistance spectrum and level were higher in pyramided lines than single resistance gene lines. M.A.S. has also been employed to improve Indian cultivars and transfer genes from pyramided lines (Sanchez *et al.*, 2000; Singh *et al.*, 2001). Vast genetic diversity is seen in wild relatives of domesticated plants. Like wild rice relatives in the genus *Oryza*, these wild rice cousins give a wealth of information on variety's origins and valuable resources for future breeding. In order to assist developing countries in bridging the gap between national research and breeding applications, Challenge Programs were established. To search for genetic diversity over a broad spectrum of germplasm resources. Backcrossing exotic cultivars or wild relatives into a superior cultivar or breeding line is a method of improving a cultivar or breeding line. Beneficial genes or alleles have been discovered in wild rice species due to backcrossing to elite cultivars (Moncada *et al.*, 2001).

Similarly, even if the parent cultivar has a superior phenotype, this method can identify foreign cultivar genes that improve phenotypically. Rice varieties are frequently cultivated for adaptability, consistency, and grain quality, making this method attractive. It was inserted into Minghui 63, a popular parent for hybrid rice production in China (Chen *et al.*, 2000). Two QTLs influencing rice yellow mottle virus resistance were inserted into IR64 (Ahmadi *et al.*, 2001). Only a tiny number of accessions can be sampled. The cost of markers has limited their usage in traditional breeding facilities for specific traits like grain quality. Low-cost marker technologies and large-scale gene discoveries will increase M.A.S. application during the next decade.

Genetic engineering: Genetic engineering is the process of altering an organism's genetic makeup via recombinant D.N.A. (rDNA). Humans have long managed to breed and select favored children in order to influence genes indirectly.

Genetic engineering is the process of altering one or more genes in a living organism. Genes from multiple species are frequently found in the genome of a single organism. It is possible to introduce genes from different biological systems into the rice plant using rice transformation techniques. Rice transformation is accomplished by using direct D.N.A. transfer methods such as protoplast-based (Datta *et al.*, 1990), biolistic, and *Agrobacterium*-mediated transformations, among others (Hei *et al.*, 1994). Rice breeding should be focused on disease and pest resistance in order to improve yield. In 1987, poisonous genes from the bacterium *Bacillus Thuringiensis* (B.T.) were inserted into the tomato, tobacco, and potato plants to protect them against aphids. When it comes to transgenic rice, the yellow stem borer is a key target for B.T. This insect is responsible for significant crop losses throughout Asia.

Selected enhanced rice cultivars are either susceptible or resistant to the bug, depending on their genetic background. As a result, B.T. transgenic rice may aid in the reduction of stem borers. These codon-optimized B.T. genes have demonstrated excellent resistance (Datta *et al.*, 1997). Trained in the field, B.T. rice has also been field-tested in China (Tu *et al.*, 2000), highly resistant to yellow stem borer populations. The study of insect resistance to proteinase inhibitors, amylase inhibitors, and lectins is becoming more popular and the study of B.T. genes. The proteolytic and hydrolytic enzymes used by insects to break down proteins and other dietary components are known as proteolytic and hydrolytic enzymes. Proteinase inhibitors (also known as -amylase inhibitors) are a natural plant defense mechanism against insect predation that must be present. Transgenic rice containing a cowpea trypsin inhibitor increased resistance to striped and pink stem borer (Xu *et al.*, 1996). The yield of rice is impacted by certain viral diseases, such as tobacco mosaic virus in tobacco and tomato, which have been effectively protected against coat protein (C.P.) mediated protection. Electroporation introduced a coat protein gene from the rice stripe virus into two japonica cultivars (Hayakawa *et al.*, 1992). Transgenic plants produced C.P. and were virus-resistant, and this trait was passed on to the progeny of the plants.

Genome editing Technologies: Increased yields and resistance to environmental stress are required to address significant issues such as population increase, climate change, and food scarcity (Clarke *et al.*, 2013; Ansari *et al.*, 2017). Although rice has been genetically engineered, it has not reached consumers due to societal and political concerns. Finally, genome engineering technologies allow in vivo D.N.A. sequence changes, which increase crop improvement potential (Figure 1). CRISPR-associated protein is a protein that is linked to CRISPR. In recent years, Cas9 (CRISPR-Cas9) has revolutionized genome engineering and emerged as a groundbreaking tool for genome editing. Other genome editing techniques, like zinc finger nucleases and TALENs,

are less versatile and simple than this method (Ma *et al.*, 2015). CRISPR-Cas9 is a low-cost, simple-to-use, highly accurate gene-editing system (Wang *et al.*, 2017). To alter many genes in different genomic regions, multiplex genome editing is required. CRISPR-Cas9 exploits double-stranded breaks (D.S.B.s) at specific locations to alter genes. The host repair process generates genomic changes, gene knockouts, and gene insertions by homology-directed repair (HDR) or non-homologous end joining (NHEJ). The host may repair without a donor template in NHEJ, while a donor template is necessary for HDR. Because of the lower frequency, HDR in plants is difficult (Puchta, 2004). CRISPR-Cas9 has a variety of applications, including plant genome editing, which is particularly useful in rice. It is an attractive paradigm for functional genomics research due to its modest genome size and close syntenic links to other cereal crops. As a result, rice genome-editing technologies such as CRISPR-Cas9 and base editing are still in the early stages of research (Li *et al.*, 2012).

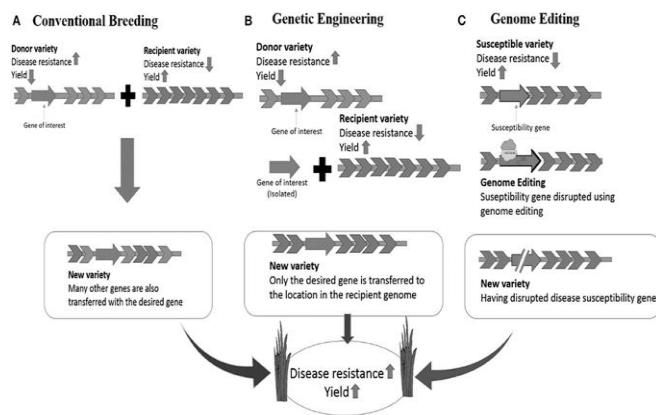


Figure 1. Comparing conventional breeding, genome editing, and genetic engineering in the development of disease-resistant rice.

CRISPR-CAS9: A new study has found base editors and prime editors that may change the rice genome, in addition to traditional genome editing that requires D.S.B. formation and relies on the NHEJ or HDR pathway for repair. The specificity of Cas9-directed D.N.A. cleavage is determined by the base pairing of the single-guide R.N.A. (sgRNA). To generate a D.S.B. at the target site (Fig. 2), Cas9 needs the PAM NGG, a nucleotide triplet (Cong *et al.*, 2013). In plants, this approach is practical. The CRISPR-Cas9 system targets the ROC5, Y.S.A., and SPP genes in rice for proof-of-concept, resulting in albino plants (Feng *et al.*, 2013). Multiplex genome editing must focus on the same or different loci in different genes. Rice utilizes tRNA processing to produce several gRNAs from a single gene, allowing for multiplex genome editing. Each gRNA is connected to the target spacer region, Endogenous RNaseP and RNaseZ processes, and cleave P.T.G. in this system's tandem arrayed tRNA–gRNA architecture. Cas9 releases several gRNAs with

the proper sequence to modify various sites in the genome (Xie *et al.*, 2015). The HDR donor repair template was likewise supplied using this tRNA processing technique (D.R.T.). For both applications, sgRNA was employed. This sgRNA contains DSB-inducing sequences as well as a D.R.T. that drives HDR. To make chimeric sgRNA, researchers used Pre-tRNA—D.R.T.—Terminator. This approach increased herbicide resistance in rice by using targeted genome editing (Butt *et al.*, 2017).

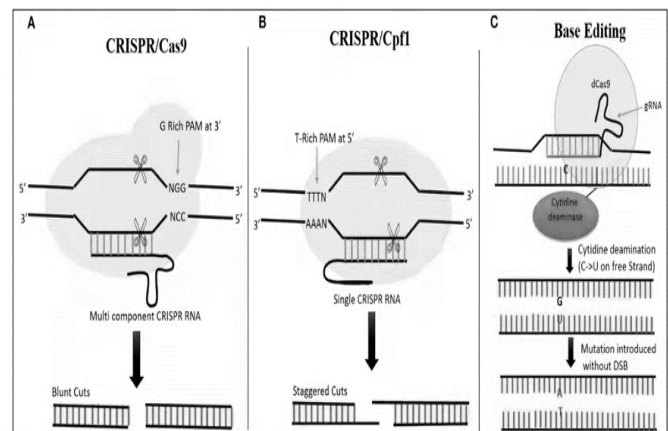


Figure 2. CRISPR/Cas9, CRISPR/Cas12a, and Base Editing Comparison.

Conclusion: Improving global rice output requires increasing rice yield potential. Conventional breeding is still used to create crop types with higher yield potential. Molecular marker-assisted breeding, genetic engineering, genome editing technologies, and CRISPR-Cas9 are just a few approaches to improve yield potential. It is also a crucial breeding technique for increasing rice production when it comes to changing plant architecture. To employ M.A.S. for trait improvement, numerous genes have been molecularly marked. Map-based cloning has identified genes for stress resistance and yield-related traits.

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Conceived the idea, designed the study, analyzed data and wrote the original article; Gull S:

Designed the study, analyzed data and writing - review & editing; Shah AZ, Faheem M, Saeed A, Khan I, Miah AAA: Writing - review & editing; Zhu X, Zhu M: Supervision

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